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OBSERVATIONS ON THE EFFECT OF SOME GLYCOSIDES OF THE DIGITALIS GROUP ON THE THYROID

EXPERIMENTAL STUDY ON GUINEA PIGS

BY

A. N. KUUSISTO

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HELSINKI, FINLAND

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PREFACE

I feel pleasure in acknowledging my indebtedness to Pentti I. Halonen, M.D., Docent at Helsinki University, Head of the Wihuri Research Institute, for valuable advice given during my work which he directed with unfailing interest and without grudging his time.

To Professor Unto Uotila, M.D., Head of the Institute of Forensic Medicine, Helsinki University, and Professor Armas Vartiainen, M.D., Head of the Institute of Pharmacology, Helsinki University, who followed and directed the work from the start and readily gave their advice, I express my deep gratitude.

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Finally I wish to thank my wife for helping me in various ways throughout the work.

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Helsinki, July 1952.

A. N. Kuusisto.

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INTRODUCTION

Since the effects of the leaf of *Digitalis purpurea* were first recognized by Withering (1785), digitalis has been the most important drug in the treatment of congestive heart failure.

Numerous clinical studies and animal tests have been carried out to explain the mechanism of the effect of digitalis. In this work interest has mainly been focussed on the effect of the drug on the heart, which has been regarded as specific, while less attention has been paid to the changes caused by digitalis in the heart and other organs.

Klopotovski (1901), who seems to have been the first to describe myocardiac necrosis caused by digitalis, also discovered that the drug gave rise to degenerative changes in the liver and the kidneys. — Hueper and Ichniowski (1941) reported on pathological changes caused by digitalis poisoning in different organs — the heart, lungs, spleen, brain, pancreas, intestines, stomach, genitals, bone marrow, uterus, lymph nodes, etc. They used cats, guinea pigs and frogs as test animals.

Before the invention of iodine treatment Crile (1922) gave digitalis and plenty of water to his patients with thyrotoxicosis, pre- and post-operatively, thus reducing considerably the mortality from the resection of the thyroid. He attributed the thyrotoxic symptoms to acute intracellular acidosis caused by increased metabolism. The organism requires an abundance of water to dilute and carry off acid residue. If the heart is sufficiently strong to provide the cells of the organism with enough water and oxygen, all is well, but if the heart fails in capacity, acidosis follows. Crile gave digitalis to all the patients as a matter of routine, apparently even to those who had not yet exhibited any congestive heart failure. The pulse of the patients treated in this way slowed down, their temperature fell, their breathing became more even, and their health in general improved.

Niccolini (1925) noted synergetic effects from the use of the thyroid hormone and digitalis. He used dogs and rats for the tests. When the dog was given digitalis or thyroid extract intravenously, the carotid pressure rose in both instances, while the pulse frequency of the heart fell. If both drugs were given simultaneously, the rise of the carotid pressure and the fall in pulse frequency were more obvious than what would have been obtained by using the doses separately. The pulse pressure, normally 2 mm Hg., after administration of digitalis 2.5 mm Hg. and of thyroid extract 1.5 mm Hg., rose to 3.5 mm Hg., when the two were given together. In tests made on the isolated heart of the rat Niccolini noted that the heart of a strongly hyperthyroid animal was insensitive to digitalis; in moderate hyperthyroidism the sensitivity to digitalis decreased somewhat, while slight hyperthyroidism temporarily increased the heart's sensibility to the drug. The removal of the thyroid enhanced the sensibility to digitalis for a time. König (1928) also noted an increase in the effect of digitalis on the isolated heart of the guinea pig if the animal's thyroid had been removed before (in his cases 18—35 days earlier).

Other work dealing with the subject has been published. Braun (1929) experimented with hyperthyroid cats and frogs and inferred that the hyperthyroid cat tolerated more digitalis, given in the form of infusions, than the control animal, before its heart stopped at systole. The isolated heart (Straub's method) of the hyperthyroid frog beat longer when treated with strophanthin than that of the control animal. — Stoye (1930) noted in the experiments made (by Langendorff's method) with the isolated heart preparations of hyperthyroid guinea pigs that larger concentrations of digitalis (1:1000 Digipurat) caused no noticeable regular change in the effect of the drug. Its toxic effect may have increased slightly. In a weaker solution (1:2000) the therapeutic effect of digitalis obviously increased. — Lendle (1933) found that thyroxin reduced the sensibility to digitalis of frogs. He observed this on the isolated heart and through determinations of the lethal dose.

Kinsell and his collaborators (1942) concluded that both »therapeutic» doses of cardiac glycoside and the hormone of the adrenal cortex, Cortin, prevented the decrease in the weight of guinea pigs which had been caused by thyroxin. Larger doses of cardiac glycoside did not prevent emaciation, but sometimes even increased

it. The writers had made a postmortem histological examination of the thyroids and the adrenals. The animals, which had been given digitalis only (a mixture of the crystalline lanatosides A, B, and C), or k-Strophanthin, did not differ from the control series essentially. The animals which had received thyroxin had lost some or all of their perirenal fat. As regards the perirenal fat, the animals which had been given thyroxin + heart glycosides did not differ to any noteworthy degree from those which had received only thyroxin. Kinsel and his collaborators therefore attributed the difference in weight between the thyroxin group and the thyroxin + cardiac glycoside group mentioned earlier to a lessened breaking-down of muscular tissue or to tissue fluid accumulation. On examining the thyroid histologically they stated: »We have so far been unable to establish any common denominator for histologic changes in the thyroid glands, either on the basis of staining properties, cell height, or colloid accumulation. Marked but inconstant variations were observed in the glands of some of the thyroxin-cortin and thyroxin-digitalis treated animals.»

THE PROBLEMS

The original purpose of the present work was to study myocardiac necrosis caused by digitalis intoxication. With a view to this, preliminary tests were made on 5 guinea pigs, on 3 of which chronic digitalis poisoning was induced in the course of 2—3 months, and digitalis was ultimately given in such large doses (4 mg/kg Digilan per os) that the animals died. Besides the heart, the other organs were also taken for a histologic examination. On examination the thyroids of the guinea pigs treated with digitalis seemed to differ from those of the 2 control guinea pigs. The follicles were larger and the epithelium flatter. There were no signs indicative of active gland function. This seemed interesting especially as there are references in the literature quoted suggesting interrelation between the thyroid hormone and the cardiac glycosides. Yet no mention is made of any thyroid changes caused by heart glycosides. Since the present writer had not met with any references dealing specifically with this question, either among the monographs on the thyroid or the cardiac glycosides or in other literature, he decided to attempt elucidation of the following problems:

1. Can the cardiac glycosides be proved to cause changes in the histologic picture of the thyroid gland?
2. If so, what are the changes like?
3. Does the administration of cardiac glycosides induce changes in the oxygen consumption and the blood cholesterol of the test animals?
4. In case the cardiac glycosides do affect the thyroid gland, along what channels does this effect take place?

MATERIAL AND METHODS

Test Animals. — One hundred and thirteen guinea pigs, 101 of them males and 12 females, were used for the tests. They were all of the same strain and of about same age, and their weight varied between 350 and 550 grams. A few larger guinea pigs, however, were also used. An attempt was made to have animals of equal weight in the corresponding test and control groups. All the test animals were kept under similar conditions in the same room, where temperature varied between 18 ° and 22 °C. There was only one control animal and one guinea pig from each test group in each cage.

Particular attention was paid to the food because certain dietary factors have been found to produce changes in thyroid structure. The observations made by Chesney and his collaborators (1928) first implied that goitre may also develop as the result of positive goitrogens in the diet. A diet consisting too predominantly of cabbage, or also of certain other vegetables, has been noted to act goitrogenically (Marine and his collaborators 1929, Astwood 1949). Marine and his collaborators (1933) found that the hyperplasia in the thyroid gland caused by cabbage (or other species of Brassica) could be prevented by giving the animals fresh grass or juice extracted from fresh vegetables. The writers believed that vitamin C in fresh vegetables might well be the antigoitrogenic factor.

With this in mind, the guinea pigs were given no cabbage. The food consisted mainly of oats with fresh grass or hay. Beetroot, carrots and turnip were also given for a change. All the animals had the same food.

Before each series of tests the guinea pigs were kept under test conditions for about two weeks so that factors of environment affecting the thyroid gland might be as nearly as possible the same.

After the tests the guinea pigs were killed by opening the chest cavity under ether anaesthesia.

Substances Studied. — The following cardiac glycosides were used: A mixture of crystalline lanatosides A, B, and C(» Digilan Orion»), crystalline digitoxin (»Digitoxin Star»), k-strophanthin (»k-Strophanthin Orion») and crystalline convallatoxin (»Convallatoxin Roche»).

The glycosides were dissolved in alcohol and then diluted with water, so that the volume percentage of alcohol was 33 in the digitoxin and 20 in the others. The glycoside consentration was 1 mg/ml.

The same solvent was used for checking the test.

The N. V. Organon »Ambinon» preparation, which the makers state contains 100 guinea pig units (Heyl-Laqueur) per ampoule, was used for the thyrotropic hormone. The Ambinon was diluted with normal saline in the ratio of 1 cc to 1 guinea pig unit.

The drugs were administered either per os or intraperitoneally. By mouth they were given by working a thin, ballheaded metal catheter through the mouth into the stomach. The strictest precautions were taken for sterilization when injecting the solution intraperitoneally. So that the doses might be exact a tuberculin syringe was used.

Electrocardiography. — To get some idea of the degree of digitalization, an electrocardiogram was taken of 10 guinea pigs after and 58 guinea pigs both before and after the treatment. Five unipolar chest leads were made by the Goldberger method as well as the ordinary limb leads. For chest leads we chose the places used by Vartio and Halonen (1950). Fig. 1.

The electrocardiogram was taken under ether anaesthesia to avoid disturbances caused by muscular tension.

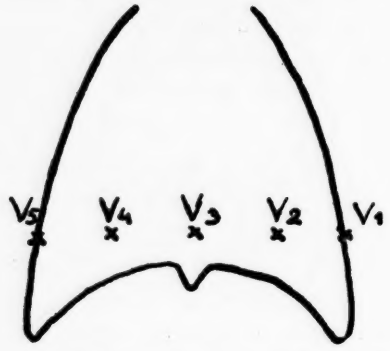


Fig. 1. — Points for electrodes in unipolar chest leads.

Studies of Oxygen Consumption. — To estimate the consumption of oxygen 5 guinea pigs were given Digilan and 5 the control solution. The determination was made by the so-called closed method, the carbon dioxide being absorbed in a 5 per cent solution of sodium hydroxide in accordance with D'Amour's and Blood's directions.

The animals were kept in a metabolism chamber before the tests until the temperature there had become steady. The temperature in the chamber averaged about 25° C, and was measured on a mercury thermometer graded with 0.1 °C accuracy. The oxygen consumption under normal conditions (760 mm Hg., 273 Abs. temp.) was estimated by corrective calculations.

For a fortnight before the actual tests the animals were often kept for acclimatization in the metabolism chamber, and normal values were taken. The actual determinations were made every fifth day on an average, for two months. Before each determination the animals were kept without food for 12 hours. At least two determinations were made each time, and the lower value for oxygen consumption was used. Whenever the animals were restless at the time, the test was scrapped and another one made.

Determination of Cholesterol. — A cholesterol determination was carried out on the blood of the same animals that were studied for oxygen consumption.

The determinations were always made 2—3 days after the oxygen determinations and extended over a period of 2 months. The guinea pigs were given no food for 12 hours before the blood samples were taken. These were obtained from the lobe of the guinea pig's ear by first making a small incision. 0.2 cc of the blood was taken in a pipette and the total cholesterol in it was obtained by Bloor's method (1916) with the Sackett modification (1925).

Histological Technique. — As soon as the animals had been killed, the thyroid glands were extracted carefully and put in a fixative. The glands of the 5 guinea pigs used for the preliminary tests were fixed in a 1:10 formol solution. The rest of the thyroids were fixed by Helly's Zenker-Formol method. The most suitable fixation time by the Helly method was found to be 2 hours. After the fixation the thyroids were rinsed by suspending them under running water for 12 hours.

The sublimate crystals were removed from the preparations

fixed by Helly's method with an iodine-alcohol solution and a water solution of sodium-thio-sulphate according to Heidenhain.

Through the series ethyl-alcohol—n-butylalcohol the preparations were transferred to cold and then to 60 °C n-butyl-alcohol saturated with paraffin and ultimately into paraffin. They were embedded in paraffin (melting point 54 °C) to which a twentieth part of beeswax had been added.

To study the histologic structure, 3—5 μ sections were cut from the one lobe of the thyroid gland, which were stained with Delafield's haematoxylin-eosin or by the method introduced by Bensley (1916), where brasilin + phosphotungstic acid and wasserblau + phosphomolybdic acid is used.

Wahlberg (1933), when employing the latter staining, noted that the blue colour of the wasserblau faded considerably if the preparations were rinsed in water, for which reason rinsing was omitted in the present work as well.

The preparations required for the measurements were taken from the other lobe of the thyroid gland. To facilitate a comparison of the preparations sections should be taken from corresponding points of each segment. For this purpose serial sections at 5 μ were taken from the whole lobe and the number of the sections was divided by 4, after which sections corresponding to points ¼, ½ and ¾ were obtained.

All the preparations used for the measurements were stained by Mallory's Azan method as modified by Koneff (1938).

Measurements Used for an Estimation of the Thyroid Function. — It is generally known that the epithelium is flattened in an inactive thyroid and the follicles contain an abundance of colloid. When the thyroid is activated, the epithelial cells become cuboidal or cylindrical, mitosis increases, the mithochondrias increase in number and size, the nuclei which in a thin epithelium seem flat in the section become more spherical and grow in size, and the amount of colloid in the follicles decreases.

To measure, in some way, the activity of the thyroid from the histologic preparations, particular attention has usually been given to the above points.

The measuring of the average height of the epithelium cells is among the best-known methods (Rawson and Starr 1938). The height of an average-sized cell from the epithelium of 200 follicles

is measured with an ocular micrometer, using oil immersion. The average height of the follicle cells is then calculated from these 200 readings.

Uotila and Kannas (1952) have developed a very practical and objective mode of measurement. In this method the preparation is reflected through the microscope onto a sheet of white paper on which a set of lines or for example 2 lines of a definite length and crossing each other obliquely have been drawn. The preparation is shifted in the microscope so as to make the lines cross the picture. The amount of epithelium, colloid and connective tissue is measured with a millimetre-measure along these lines. When the measurements are made on tissue from a number of points in the thyroid, the relation between the epithelium, the colloid and the stroma is obtained with considerable accuracy and can be expressed in percentages.

Tala (1952) checked the accuracy of the method by comparing the values obtained in the manner just described with those he got by drawing the same reflected figure on paper and cutting out the colloid, the epithelium and the stroma and then weighing the bits of paper. The values obtained by measurement differed from those obtained by weighing by ± 0.5 per cent or less.

In the present work, in the first series of tests, the methods of Rawson and Starr, and Uotila and Kannas were used side by side. In the later series only the latter was employed. The preparation from the thyroid was reflected with a magnification of 400 (objective 10/1 and planocular No. 8) on paper and then measured along two straight 40 cm long lines which crossed each other obliquely. Two measurements of this kind were made in adjacent areas of the section taken from the centre of the lobe, while one measurement was made in the sections taken from the centre of each half-lobe. The measurements were performed at the approximate centre of the section. Large strands of connective tissue which happened to run along the line of measurement were avoided. Apart from this no selection was made of the points to be measured.

Statistical Treatment. — In the calculations n represents the number of observations, while M stands for the arithmetical mean. The standard deviation s is obtained from the following formula:

$$s = \sqrt{\frac{\Sigma (a_i - M)^2}{n - 1}},$$

where a_i is one single observation. The standard deviation m of the arithmetical mean is obtained from the formula:

$$m = \frac{s}{\sqrt{n}}.$$

To find out whether the difference between the two arithmetical means $(M_1—M_2)$ is significant, Student's t-test was applied:

$$t = \frac{M_1 - M_2}{\sqrt{\frac{(n_1 + n_2)\,[(n_1 - 1)\ s_1^2 + (n_2 - 1)\ s_2^2]}{n_1 \cdot n_2\,(n_1 + n_2 - 2)}}}$$

The completed table shows to what percentage of probability the value t obtained corresponds.

The difference is almost significant if the probability in question is between 95 and 99 per cent, significant if it is 99—99.9 per cent, and highly significant if it is above 99.9 per cent.

TESTS MADE TO DISCOVER THE EFFECT ON THE THYROID OF SOME GLYCOSIDES OF THE DIGITALIS GROUP

Tests Made with a Mixture of Lanata Glycosides (Digilan). — We decided to check the observation made in the preliminary tests on a larger number of animals. For this we took 20 male guinea pigs, 10 of which received Digilan by mouth and 10 the control solution. In the tests mentioned in the introduction, toxic doses of Digilan were used deliberately, but in this series it was decided to give Digilan in smaller doses, *i.e.* 1 mg to each kilogram of the animal's body weight. Even this was a comparatively large dose, but when given by mouth, the guinea pigs tolerate it well. The Digilan and the control solution were given once daily for 60 days. The series of experiments was carried out between Sept. 12 and Nov. 10, 1950.

In the course of the test 2 of the guinea pigs in the Digilan group died of intestinal infection and 1 of pneumonia, 1 of the control group of an intestinal infection, 1 of pneumonia and 1 of perforation of the oesophagus. These guinea pigs were not taken into account in estimating the results. At autopsy it appeared that there was very little fatty tissue in the guinea pigs of the Digilan group. The perirenal fat had almost disappeared. In other respects there were no differences visible to the naked eye between these animals and the control ones. On three digitalized guinea pigs and two control ones the mucous membrane of the stomach appeared slightly thickened and congested.

Histologic examination of the thyroid glands: In the thyroids of the control animals the size and form of the follicles varied from large spherical to small spherical or shapeless. The large follicles, which frequently appeared in the periphery of the segment, had shallow, flat epithelium, while in the smaller ones it was mainly cuboidal or sometimes high and cylindrical in form. The nuclei were

oblong in the low epithelial cells and round in the rest. In the Azan and Bensley stainings the colloid in some of the follicles was stained basofilously red and in others acidofilously blue. Sometimes the colloid in the centre of the follicle would be of the concentrated kind staining dark red while around the edge it was a thinner pale blue. In a number of follicles, especially those containing acidophil colloid, clear, bluish droplets of secretion, secreted by the cells, could be distinguished at the edge of the colloid, most of which seemed still to be suspended, as it were, from the apex of the cell. At other points on the edge of the colloid so-called resorption vacuoles might occur, small, empty cavities, whose base adhered to the cell apex on a broad surface. Slight hyperemia could sometimes be noted in the preparations with a more active appearance.

The thyroids of the control guinea pigs were histologically similar to those of normal guinea pigs described by Uotila (1934).

In the thyroid glands of the guinea pigs which were given Digilan, the follicles were obviously larger and their shape did not vary so much as in the controls. The epithelium was lower, often quite flattened. There were markedly fewer signs indicative of cellular activity, such as the droplets of secretion and the resorption vacuoles just mentioned. Nor was there any hyperemia. There was no noteworthy difference in the capacity of the colloid to stain, compared with the control series (Fig. 2a and 2b).

When measuring the average height of the epithelial cell by Rawson's and Starr's method, $4.7 \pm 0.6\ \mu$[1] was obtained as the mean value for the digitalis group and $6.1 \pm 0.5\ \mu$ for the control series. The difference between the means, $1.4\ \mu$, is not yet statistically significant, but still on the borderline. (The value obtained for t was 2.02 and the t corresponding to a 95 per cent certainty = 2.18). (Fig. 3, p. 20).

The proportions of the thyroid epithelium, colloid and stroma were measured by Uotila and Kannas' method. The amounts of epithelium are shown in Fig. 4, p. 21 and those of colloid in Fig. 5, p. 22. Since the stroma is apparently of no essential significance and since its amount can be calculated by subtracting the percentage of the epithelium and that of the colloid from 100, it has not been given separately. In the control series the mean of the epithelial

[1] Here and in the following the $\pm$ value is the standard deviation of the arithmetical mean.

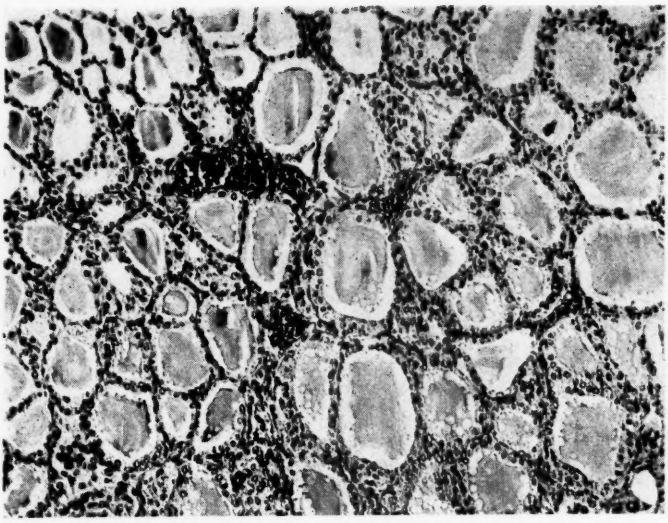


Fig. 2a. — The thyroid of a guinea pig given control solution by mouth for 60 days. Magnification × 160. Helly-Bensley staining.

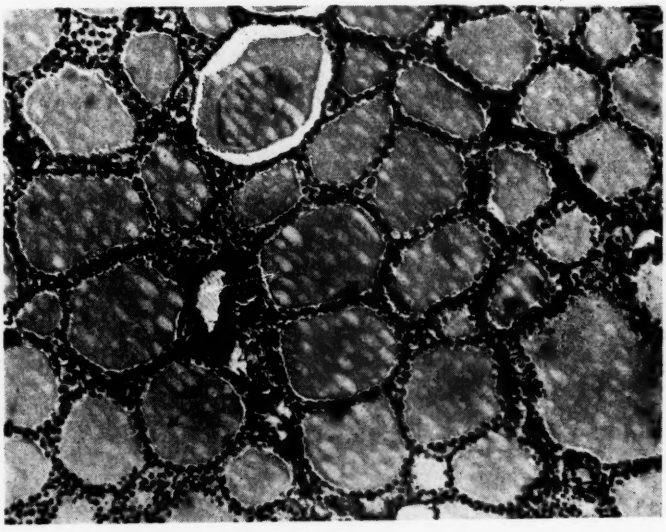


Fig. 2b. — The thyroid of a guinea pig given Digilan for 60 days. The daily Digilan dose was 1 mg/kg by mouth. Magnification × 160. Helly-Bensley staining.

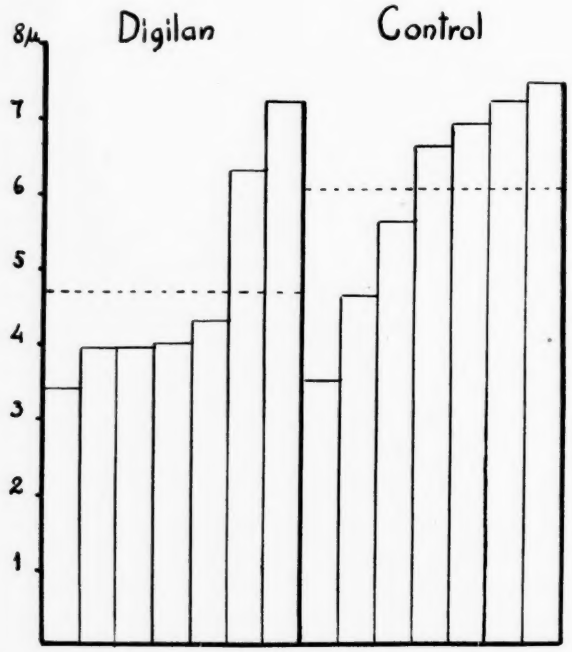


Fig. 3. — Each column represents the average height of the epithelium of one thyroid. Digilan group on the left and control group on the right, both in order of height. μ-scale is entered in the ordinate. The means are indicated by a broken line. The test took 60 days, the daily Digilan dose was 1 mg/kg per os.

proportions was 64.8 ± 1.8 per cent and in the Digilan series 41.9 ± 3 per cent. The difference between the means, 22.9 per cent, is highly significant. The mean of the colloid percentage was 22.4 ± 2.7 in the control group and in the Digilan group 50.5 ± 2.7 per cent. The difference between the means, 28.1 per cent, is statistically highly significant.

The test series seems to show that the follicular epithelium is flatter in the Digilan group than in the control one. The proportion of epithelium is smaller and that of colloid noticeably larger than in the control series. Obviously the Digilan has the effect of somehow inactivating the thyroids of the guinea pigs examined in this series of tests.

Only male guinea pigs were used in the test described above, since they have no such hormonal fluctuation as the females due to their cycle. To find out whether digitalis has a similar effect on

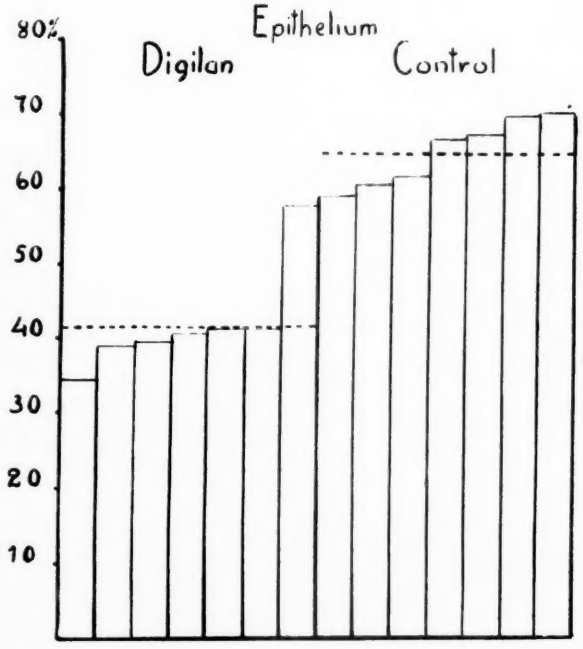


Fig. 4. — Proportions of epithelium. Each column gives the amount of epithelium contained in one thyroid, in percentages. Digilan group on the left and control group on the right, both in order of height. Epithelial percentages are entered in the ordinate. The means are indicated by a broken line. The test took 60 days, the daily dose of Digilan was 1 mg/kg per os.

the thyroid of female guinea pigs, tests were made on a small series of females as well. We took 12 non-pregnant females 6 of which were given the control solution and 6 Digilan. The substances were injected intraperitoneally once a day for 60 days. The daily dose of Digilan was 0.2 mg/kg. The lethal dose of Digilan, according to determinations made by the Lääketehdas Orion Oy., Manufacturing Chemists, was 2.85 mg/kg [the Knaffl-Lenz (1926) guinea pig method]. The tests on female guinea pigs were made between Oct. 1 and Nov. 30, 1951. The general health of the guinea pigs was good during the test.

Tables 1—2, pp. 22—23 show the weights of the guinea pigs at the beginning and the end of the test, the percentile changes in weight and the weights of the thyroids in milligrams to every 100 g of the animal's weight. In the control group the change in weight was on an average +8.1 per cent and in the Digilan group +4.6 per cent. Even in

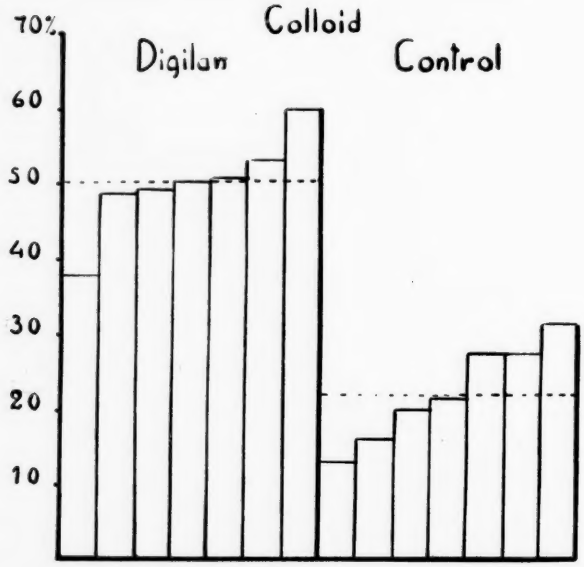


Fig. 5. — Proportions of colloid. Each column represents the amount of colloid contained in one thyroid, in percentages. Digilan group on the left, control group in the right, both in order of columnar height. Percentages are entered in the ordinate. The means are indicated by broken lines. The test took 60 days, the daily dose of Digilan was 1 mg/kg per os.

TABLE 1

FEMALE GUINEA PIGS GIVEN CONTROL SOLUTION DAILY, INTRAPERITONEALLY. THE TEST TOOK 60 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
108	450 g	500 g	+11 %	15.0 mg/100 g
109	460 »	510 »	+11 »	10.8 »
110	420 »	450 »	+ 7 »	17.8 »
111	500 »	540 »	+ 8 »	11.1 »
112	520 »	550 »	+ 6 »	16.6 »
113	520 »	550 »	+ 6 »	12.2 »
Mean	478 g	516 g	+ 8.1 %	13.9 mg/100 g

this series of tests digitalis had no appreciable effect on the weight of the thyroid.

Histologically the thyroid glands of the control series seemed less active than the earlier controls. There was more colloid and

TABLE 2

FEMALE GUINEA PIGS GIVEN 0.2 MG/KG DIGILAN DAILY, INTRAPERITONEALLY. THE TEST TOOK 60 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
102	510 g	600 g	+18 %	12.5 mg/100 g
103	590 »	625 »	+ 6 »	10.9 »
104	460 »	460 »	0 »	14.8 »
105	610 »	600 »	— 2 »	15.8 »
106	420 »	460 »	+10 »	14.5 »
107	600 »	575 »	— 4 »	10.4 »
Mean	532 g	553 g	+ 4.6 %	13.1 mg/100 g

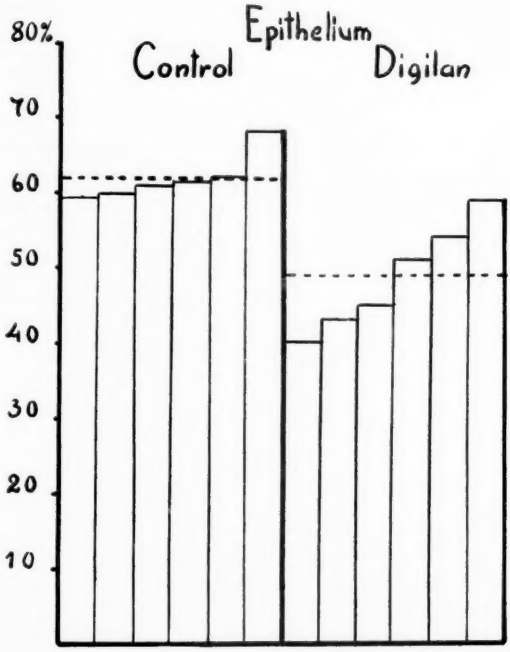


Fig. 6. — Proportions of epithelium. Each column represents the proportion of epithelium contained in one thyroid. On the left the female guinea pigs given control solution, on the right those given 0.2 mg/kg Digilan daily, intraperitoneally. The columns are in order of height in each group. The means are indicated by broken lines. The percentages are entered in the ordinate. The test took 60 days.

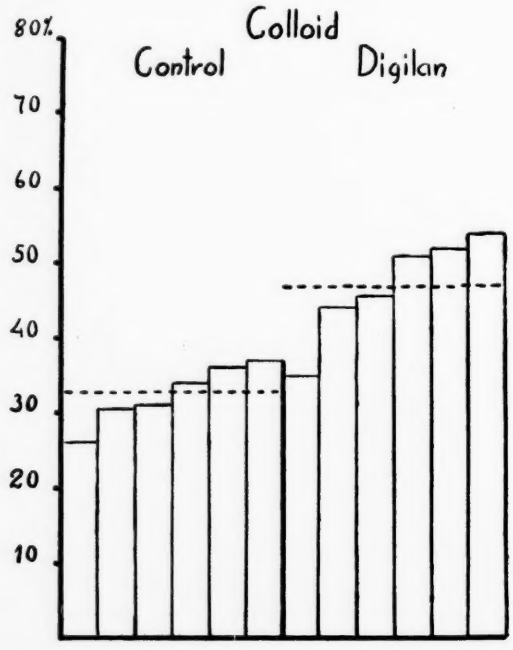


Fig. 7. — Proportions of colloid. Each column represents the proportion of colloid contained in one thyroid. On the left the female guinea pigs which were given control solution, on the right those given 0.2 mg/kg Digilan daily, intraperitoneally. The columns are in order of height, in each group. The means are indicated by broken lines. The percentages are entered in the ordinate. The test took 60 days.

the epithelium was flatter. In the glands of the Digilan group the follicles were still larger, the epithelium was flatter and there was less secretional activity in the cells. The proportions of epithelium contained in the thyroids are shown in Fig. 6, p. 23 and the amounts of colloid in Fig. 7. In the control group the mean epithelium percentage was 62.3 ± 1.5 and in the Digilan group 48.8 ± 3 per cent. The difference between the means is statistically significant. The mean of the proportions of colloid was 32.8 ± 1.8 in the control group and in the Digilan group 46.9 ± 2.8 per cent. The difference between the means is statistically significant. The Digilan thus seems to have the same inactivating effect on the thyroids of female guinea pigs as on males in the earlier test.

Experiments with Digitoxin, k-Strophanthin and Convallatoxin. — Since Digilan gave rise to such obvious changes, it was interesting to see whether other cardiac glycosides acted similarly or whether

the effect obtained was only characteristic of the lanata group. Experiments with digitoxin, k-strophanthin and convallatoxin were therefore considered justifiable.

It was assumed that relatively large but still sub-toxic doses of glycosides would serve the purpose.

The Lääketehdas Orion Oy., Manufacturing Chemists kindly determined the lethal doses to guinea pigs of the glycosides employed, by the Knaffl-Lenz method. The lethal doses were:

Digitoxin	.....	1.30 mg/kg
k-Strophanthin	.....	0.44 »
Convallatoxin	.....	0.25 »

The resistance of guinea pigs to digitalis shows additional fluctuation according to season. They are less resistant in summer than in winter, which is considered to be due partly to the temperature (Haskell 1914). It is interesting to note, in this connection, that the thyroid also manifests changes reflecting the seasons. These, too, are assumed to be traceable primarily to temperature. Heat inactivates and cold activates the gland (Mills 1918).

It is comparatively difficult to establish a suitable sub-toxic dose. With this object preliminary experiments were made with 6 male guinea pigs. The glycosides were given in the form of intraperitoneal injections, once daily between June 11 and July 10, 1951. The doses given to the guinea pigs and the changes in body weight appear in the Table 3. The guinea pigs were in good health during the test. Electrocardiograms taken before the test and after showed

TABLE 3

TO ESTABLISH A SUITABLE DOSE OF THE GLYCOSIDE THE ABOVE AMOUNTS WERE TESTED ON 30 CONSECUTIVE DAYS

No. of Animal	Cardiac Glycoside Used and its Daily Dose			Weight at Commencement	Weight at End	Change of Weight in Percentages
56	k-Strophantin	0.3	mg/kg	520 g	510 g	— 2
57	»	0.4	»	380 »	425 »	+12
58	Convallatoxin	0.1	»	505 »	470 »	— 7
59	»	0.15	»	400 »	385 »	— 4
60	Digitoxin	0.2	»	500 »	510 »	+ 2
61	»	0.3	»	470 »	455 »	— 3

no changes except in guinea pig No. 61, which had been injected with 0.3 mg/kg of digitoxin. In this Ecg, a slight fall of the S—T segment was noted in all leads, and the T-wave turned negative in leads V_2 and V_3.

I decided to employ the following doses for the actual test series: 0.2 mg/kg digitoxin, 0.2 mg/kg k-strophanthin and 0.1 mg/kg convallatoxin.

The series consisted of 40 male guinea pigs, 10 of which were given control solution, 10 digitoxin, 10 k-strophanthin and 10 convallatoxin. The drugs were injected intraperitoneally once daily for 30 days. For practical reasons the test was begun in groups, for the control group on Aug. 1, the digitoxin group on Aug. 2 the strophanthin group on Aug. 3 and the convallatoxin group on Aug. 4, 1951.

Of the animals which were given digitoxin 1 died of peritonitis. Of those treated with k-strophanthin 1 died of pneumonia during the test and 1 had to be killed before the termination of the series because it had been bitten by the other guinea pigs, and 2 of the convallatoxin-treated animals died of intestinal infection. These guinea pigs were not included in our estimate of the results. The rest of the guinea pigs were in good health throughout the tests.

Tables 4—7 give the weights of the guinea pigs at the beginning

TABLE 4

GUINEA PIGS GIVEN CONTROL SOLUTION INTRAPERITONEALLY, ONCE DAILY. THE TEST TOOK 30 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
62	400 g	460 g	+15 %	11.3 mg/100 g
66	430 »	450 »	+ 5 »	10.9 »
70	485 »	550 »	+13 »	14.4 »
74	450 »	455 »	+ 1 »	11.0 »
78	440 »	450 »	+ 2 »	12.9 »
82	450 »	485 »	+ 8 »	11.3 »
86	445 »	460 »	+ 3 »	10.9 »
90	515 »	570 »	+11 »	10.3 »
94	435 »	450 »	+ 3 »	14.9 »
98	430 »	490 »	+14 »	14.5 »
Mean	448 g	482 g	+ 7.5 %	12.2 mg/100 g

TABLE 5

GUINEA PIGS GIVEN 0.2 MG/KG DIGITOXIN DAILY, INTRAPERITONEALLY. THE TEST TOOK 30 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
64	405 g	370 g	— 9 %	14.1 mg/100 g
68	465 »	450 »	— 3 »	8.5 »
72	300 »	340 »	+13 »	11.8 »
76	380 »	440 »	+16 »	11.8 »
80	450 »	420 »	— 7 »	13.3 »
84	385 »	390 »	+ 1 »	10.0 »
88	500 »	470 »	— 6 »	13.8 »
92	420 »	380 »	—10 »	13.1 »
100	385 »	385 »	0 »	13.5 »
Mean	410 g	405 g	— 0.6 %	12.2 mg/100 g

TABLE 6

GUINEA PIGS GIVEN 0.2 MG/KG DAILY OF K-STROPHANTHIN INTRAPERITONEALLY. THE TEST TOOK 30 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
63	450 g	530 g	+18 %	12.1 mg/100 g
67	490 »	470 »	— 4 »	9.6 »
75	485 »	440 »	— 9 »	12.3 »
79	460 »	325 »	—29 »	15.4 »
91	380 »	350 »	— 8 »	12.0 »
83	400 »	350 »	—13 »	12.8 »
87	465 »	500 »	+ 8 »	15.0 »
95	470 »	510 »	+ 9 »	13.1 »
Mean	450 g	434 g	— 3.5 %	12.8 mg/100 g

of the test and at its completion, the changes in body weight in percentages and the weights of the thyroids to each 100 g of the animal's weight. The change in weight was + 7.6 per cent on an average in the control group, —0.6 per cent in the digitoxin group, —3.5 per cent in the k-strophanthin group and —6 per cent in the convallatoxin group. The thyroid weights showed no appreciable differences.

The electrocardiograms taken after the test showed no changes as compared with those taken before it.

TABLE 7

GUINEA PIGS GIVEN 0.1 MG/KG CONVALLATOXIN DAILY INTRAPERITONEALLY. THE TEST TOOK 30 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
69	500 g	450 g	—10 %	8.5 mg/100 g
73	500 »	520 »	+ 4 »	11.5 »
77	440 »	380 »	—14 »	13.7 »
81	500 »	490 »	— 2 »	12.2 »
85	470 »	420 »	—11 »	11.7 »
93	480 »	430 »	—10 »	14.0 »
97	460 »	430 »	— 7 »	10.2 »
101	350 »	360 »	+ 3 »	10.3 »
Mean	463 g	435 g	— 6 %	11.5 mg/100 g

Histologically the thyroids of the animals which had been given cardiac glycosides showed similar changes as in the previous series of tests. The epithelium appeared flatter, and there was more colloid than in the controls. The proportions of epithelium and colloid, measured by Uotila and Kannas' method, are entered in Figs. 8 and 9.

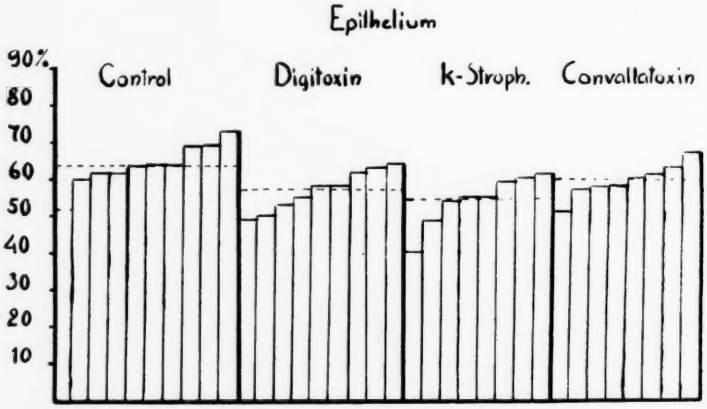


Fig. 8. — Proportions of epithelium. Each column represents the amount of epithelium contained in one thyroid. From left to right: the animals given control solution, those given 0.2 mg/kg digitoxin, 0.2 mg/kg k-strophanthin and 0.1 mg/kg convallatoxin daily. The columns are in groups in order of height. The means are indicated by broken lines. The percentages are entered in the ordinate. The test took 30 days.

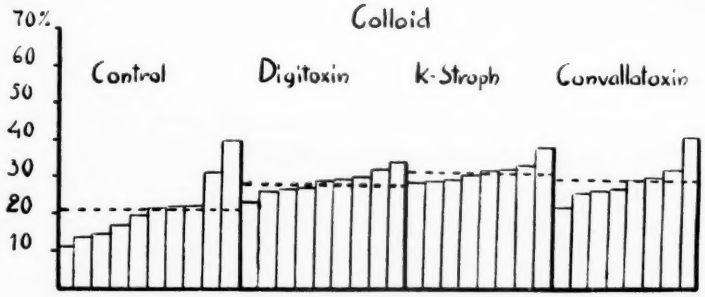


Fig. 9. — Proportions of colloid. Each column represents the amount of colloid contained in one thyroid. The animals which were given control solution on the left, then those given 0.2 mg/kg digitoxin, 0.2 mg/kg k-strophanthin and 0.1 mg/kg convallatoxin daily. The columns are in groups in order of height. The means are indicated by broken lines. The percentages are entered in the ordinate. The test took 30 days.

The mean of the proportions of epithelium was 63.9 ± 1.8 per cent in the control group, 57.2 ± 1.8 in the digitoxin group, 54.3 ± 2.4 per cent in the k-strophanthin group and in the convallatoxin group 59.7 ± 1.8 per cent. Compared with the control group the difference is significant in the digitoxin and k-strophanthin groups. There is no statistical difference between the mean proportions of epithelium of the convallatoxin and control groups. For colloid the corresponding proportions were as follows: Control group 21.2 ± 2.7 per cent, digitoxin group 28.6 ± 1.2, k-strophanthin group 31.5 ± 1.1, and convallatoxin group 29.4 ± 1.9 per cent. Compared with the control group the difference is statistically significant in the k-strophanthin group, and almost significant in the digitoxin and convallatoxin groups.

In the series of tests described above digitoxin and k-strophanthin produced the same effect on the proportions of the thyroid epithelium and colloid as the mixture of lanata glycosides reviewed previously. Convallatoxin possibly also reduces the proportion of epithelium and, with a considerable degree of certainty, increases that of colloid.

The drug last mentioned produced the least noticeable effect in the present test. This may be due to the fact that the dose used was the smallest in weight, or possibly to the fact that convallaria glycosides are eliminated from the organism much more quickly than are other cardiac glycosides (Vartiainen 1929). Nor is the

effect on the thyroid of the other substances studied so clearly apparent as in the first series of tests, but then only 30 days, half the time, was used for the experiment.

In the tests described the cardiac glycosides employed gave rise to changes indicative of inactivation of the guinea pig thyroids. The epithelium was flattened, the amount of colloid increased and the active function of the epithelium cell was reduced. Clear and statistically significant differences were obtained with the mixture of lanata glycosides, with digitoxin and k-strophanthin. Convallatoxin produced effects of a similar trend, though not equally clear. — The doses of glycoside used were large, but may yet be considered sub-toxic. The animals tolerated them comparatively well, and they caused no changes on the electrocardiograms. In comparison with the controls, the animals usually lost some weight. The emaciation was most marked in the convallatoxin group where relatively large doses were given, though in weight they were only half that of the other glycoside doses.

DETERMINATIONS OF OXYGEN CONSUMPTION AND BLOOD CHOLESTEROL

The tests described showed that digitalis has an inactivating effect on thyroid function. It is therefore interesting to see whether digitalis in any way affects the guinea pig's oxygen consumption and blood cholesterol, which are known to be partly contingent on the function of the thyroid.

Several workers have noted an increase in the basal metabolic rate of patients suffering from congestive heart failure (Peabody and collaborators 1916, Peabody and collaborators 1917, Peabody and Sturgis 1922). It is known also that when the heart failure is treated basal metabolism falls (Nylin 1933, Strieck and Marble 1935, Resnik and Friedman 1935). Nylin (1938) studied the effect of digitalis on the basal metabolism of patients with congestive heart failure and wrote:

»1. In cases of considerable cardiac insufficiency with congestion, swelling of the liver and anasarca, the standard metabolism has been found to be appreciably increased.

2. In cases without cirrhosis of the liver, »Digiton» in large doses gives rise to a momentary further increase in the standard metabolism and blood pressure and a reduction in the heart rate, followed later by a considerable reduction in the standard metabolism and blood pressure. Associated with these changes there is an improvement in the patient, with reduction of the size of the swollen liver and considerable relief from edema.

3. In cases of cardiac insufficiency with cirrhosis of the liver, digitalization does not seem to cause a momentary increase in the standard metabolism.»

Starr and his collaborators (1937) mentioned two cases of hyperthyroidism in which administration of digitalis reduced the oxygen consumption. — Digitalis obviously does not affect the

basal metabolism and respiration of normal individuals (Anthony and collaborators 1933).

Bomskov and his collaborators (1941) noted, in experiments on guinea pigs, that small doses of digitalis prevented the basal metabolic rate, produced by the thyrotropic hormone, from increasing, whereas large doses of digitalis had an effect parallel with the thyrotropic hormone and increased the basal metabolism. Small doses of digitalis had no effect on the basal metabolism of normal guinea pigs. The writers measured the consumption of oxygen to each kilogram of the animal's body weight and used this for measuring the basal metabolism.

Choisy (1930) studied the effect of digitalis on the blood cholesterol and noted a reduction when patients with congestive heart failure were given therapeutic doses of digitalis, and it remained reduced while the digitalization was continued. The lowest cholesterol value was obtained about 3—4 hours after the administration of the drug. Two patients with digitalis intoxication on entry to hospital had a very low blood cholesterol which rose when the intoxication disappeared. The author mentions another patient with a low blood cholesterol value, 0.87 per cent, on admission, which rose in three days to 1.71 per cent. In experiments on rabbits he found that digitalis reduced the blood cholesterol temporarily. If the animal was given digitalis daily, the cholesterol decreased within about 3—4 days and then after some time exceeded the initial values, returning gradually to normal. Choisy used a preparation free from saponins (Digitalin Nativelle) for his experiments.

As has been previously stated (Chapter III, p. 13), the oxygen consumption and the blood cholesterol of 10 male guinea pigs was determined.

Five of these were given Digilan and 5 control solution by mouth. The test took 70 days and was carried out between Jan. 27 and Apr. 6, 1951. The daily dose of Digilan was 1 mg/kg for the first 30 days, but was raised to 2 mg/kg for the rest of the time. The guinea pigs were in good health throughout the test. The electrocardiograms taken after the test showed no pathological changes[1]. The body

[1] In this series a histological examination was made of the heart and liver preparations of the guinea pigs. It was found that the dose of digitalis used caused no pathological changes on them.

TABLE 8

CONTROL GUINEA PIGS USED FOR OXYGEN CONSUMPTION AND CHOLESTEROL DETERMINATIONS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
27	415 g	375 g	—10 %	26.7 mg/100 g
29	365 »	335 »	— 8 »	14.9 »
31	495 »	505 »	+ 2 »	19.8 »
33	470 »	530 »	+13 »	23.3 »
35	385 »	390 »	+ 1 »	19.2 »
Mean	426 g	427 g	— 0.4%	20.8 mg/100 g

TABLE 9

GUINEA PIGS USED FOR OXYGEN CONSUMPTION AND CHOLESTEROL DETERMINATIONS, WHICH WERE GIVEN DIGILAN BY MOUTH FOR 70 DAYS. THE DAILY DOSE OF DIGILAN WAS 1 MG/KG FOR THE FIRST 30 DAYS, AFTER WHICH THE DOSE WAS DOUBLED

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
26	440 g	440 g	0 %	11.4 mg/100 g
28	450 »	435 »	— 3 »	19.5 »
30	520 »	490 »	— 6 »	20.4 »
32	360 »	300 »	—17 »	23.4 »
34	415 »	410 »	— 1 »	25.6 »
Mean	437 g	415 g	— 5.4%	20.6 mg/100 g

weights of the guinea pigs at the beginning and end of the test, the changes of their weights in percentages and their thyroid weights to every 100 g of the animal's body weight appear in Tables 8 and 9. The average change in body weight was —0.4 per cent in the control group and —5.4 per cent in the Digilan group. There were no appreciable changes in the weights of the thyroids.

No appreciable differences were noted in oxygen consumption between the control and the Digilan groups. Twelve determinations were made on each guinea pig during the test. In the control group the average oxygen consumption was 852 $\pm$ 12.5 cc an hour to a kilogram of the animal's weight. For the Digilan group the

corresponding value was 865 ± 14.5 cc. Statistically there is no difference between the means of the oxygen consumption. Nor were there any statistically significant differences in determinations made on different days between the control group and the group given Digilan.

On examining the effect of digitalis on the blood cholesterol it was found to have fallen slightly during the digitalization. Two determinations were made on all the guinea pigs before administration of digitalis. The mean of the value obtained for these 20 normal determinations may be regarded roughly as the mean blood cholesterol value of these guinea pigs. It was 163 ± 4.5 mg per 100 ml. The values were determined 12 times on each of the guinea pigs in the course of the test. Some specimens were spoiled, but 50 were satisfactory in the control group and 46 in the Digilan group. The mean value for those which were given control solution (20 per cent alcohol) was 163.6 ± 2.6 mg per 100 ml. The control solution thus caused no change on the mean value of the estimates made during the test. The mean during the test of the blood cholesterol of the digitalized guinea pigs was 149.2 ± 2.4 mg per 100 ml. The difference in comparison with the mean of the control group is 14.4 mg per 100 ml, which is statistically significant. The results are presented graphically (Fig. 10). The graph shows that 3 and 6 days after the beginning of digitalization the cholesterol of the digitalized guinea pigs was appreciably lower than the initial value. The difference between the mean of these determinations and the mean of the initial values is 22.4 mg per 100 ml, which is statistically significant. These low values were followed by a compensatory rise which was noted 9 days after the beginning of the test. The difference as compared with the low values is statistically significant. After this the blood cholesterol fell again. As regards digitalis alone, the tests are thus in agreement with Choisy's findings, but, as appears from the graph, there was a fall and afterwards a compensatory elevation in the blood cholesterol of the control group also. It is true that these changes are not so clear in the control group nor are the differences statistically significant compared with the initial values, but there is no statistically significant difference between the digitalis group and the control group in the determinations made after 3, 6 and 9 days. Choisy used no control animals to check his tests. It is not impossible that the sharp fall and the

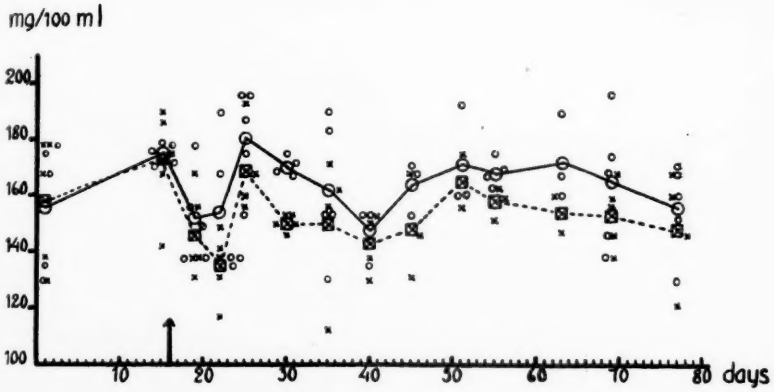


Fig. 10. — Graphic presentation of the blood cholesterol values of the guinea pigs which were given control solution and Digilan by mouth for 70 days. The cholesterol in mg per 100 ml is entered in the ordinate and the time in the absciss. o = single cholesterol value of a control guinea pig. ○ = mean of the cholesterol values determined on the control guinea pigs simultaneously. These means are connected by a solid line. One single observation made of a guinea pig given digitalis = x, mean of simultaneous determinations = ⊠. These means are connected by a broken line. ↑ = beginning of the administration of Digilan.

subsequent compensatory rise in the blood cholesterol may be due to the solvent, or the handling of the animals only, and that digitalization merely helps to keep the blood cholesterol slightly below normal level.

The thyroids of the animals employed for the series of tests were examined histologically and the amounts of epithelium and colloid were measured as before. As in the previous tests changes with a similar trend were revealed in the thyroids. The proportions of the epithelium and the colloid are presented in Figs. 11 and 12, p. 36. The mean of the proportions of epithelium was 54.4 ± 4.2 per cent in the control group and 42.6 ± 3.2 in the Digilan group. The difference between the means is not yet statistically significant, but approaches 95 per cent probability (t obtained $= 2.25$, the t corresponding to 95 per cent probability $= 2.31$). The corresponding values for colloid were in the control group 20 ± 1.8 per cent and in the Digilan group 41.5 ± 2.1 per cent. The difference between the means is highly significant.

This test series showed that digitalis produced no such change in the oxygen consumption of the guinea pigs used for the tests

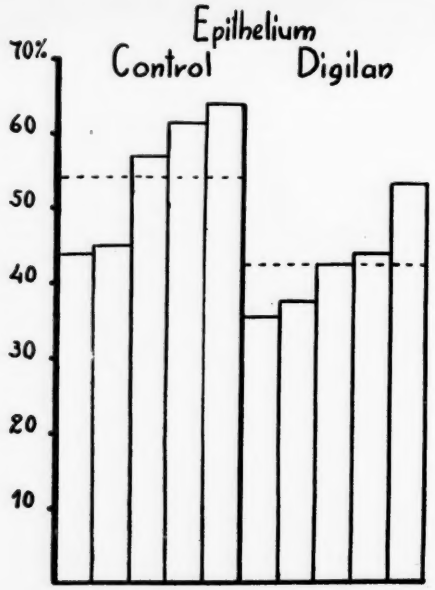


Fig. 11. — Proportions of epithelium. Each column represents the proportion of epithelium contained in one thyroid. On the left the control group, on the right the Digilan group. The daily dose of Digilan was 1 mg/kg per os for 30 days, and afterwards 2 mg/kg for 40 days. The columns are in order of height in each group. The means are indicated by broken lines. The percentages are entered in the ordinate.

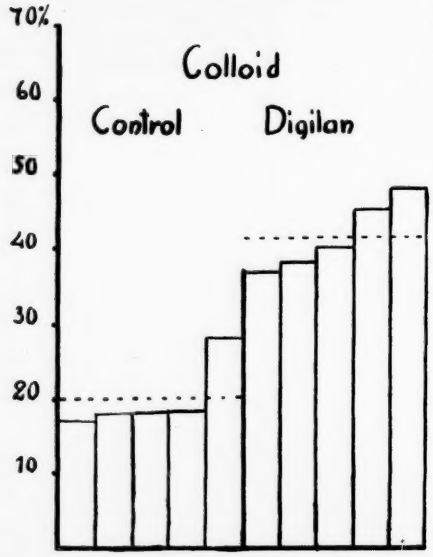


Fig. 12. — Proportions of colloid. Each column represents the percentile amount of colloid contained in one thyroid. On the left the controls, on the right the Digilan group. The daily dose of Digilan was 1 mg/kg for 30 days and afterwards 2 mg/kg for 40 days. The columns are in order of height in both groups. The means are indicated by broken lines. The percentages are entered in the ordinate.

— at least such as was ascertainable by the method employed. Bomskov and his collaborators also failed to notice that any change was effected in the oxygen consumption of normal guinea pigs by administration of digitalis.

Doses of digitalis reduced the blood cholesterol of the guinea pigs used for the tests. The cholesterol first fell markedly, the specimens taken 9 days after the beginning of the digitalization were again on a level with the initial values and afterwards declined. Similar fluctuation, though less clear, occurred also in the blood cholesterol values of the control group.

Digitalis had a similar effect on the thyroids as in the previous tests. The proportion of the epithelium apparently fell and the amount of colloid increased clearly.

EFFECT OF LANATA GLYCOSIDES ON THYROID ACTIVATED BY THE THYROTROPIC HORMONE

In the preceding chapter reference was made to the discovery by Bomskov and his collaborators that comparatively small doses of digitalis are capable of reducing the basal metabolic rate produced by the thyrotropic hormone. They paid, however, no notice to the histology of the thyroid. It is therefore interesting to see whether digitalis is capable of preventing the activating effect of the thyrotropic hormone on the thyroid.

We took 20 guinea pigs, 5 of which were given control solution, 5 Digilan (»Digilan Orion»), 5 thyrotropic hormone and 5 thyrotropic hormone + Digilan. The substances were injected intraperitoneally once daily. The Digilan dose was 0.2 mg/kg each time and that of the thyrotropic hormone $^1/_{10}$ guinea pig units (Heyl-Laqueur). The injections were given for 9 days beginning on June 11, 1951. The animals were killed on June 20. It is not customary to give the thyrotropic hormone over a longer period, since it is known to have no effect on the thyroid later (Collip and Anderson 1934, Werner 1936). The electrocardiograms taken before and after the test revealed no pathological changes. The guinea pigs were in good health during the test. The weights of the guinea pigs at the beginning and end of the test, the percentile changes that occurred in them and the weights of the thyroids in mg to each 100 g of the animal's body weight are given in Tables 10—13. The weights of the guinea pigs in the control group rose slightly, the average increase being 2.4 ± 0.7 per cent. The digitalized guinea pigs may have lost some weight, the average change being -0.8 ± 1.6 per cent. The dispersion is large enough to make the difference between

TABLE 10

GUINEA PIGS GIVEN CONTROL SOLUTION DAILY. THE TEST TOOK 9 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
37	480 g	500 g	+4 %	17.0 mg/100 g
41	410 »	415 »	+1 »	15.6 »
45	445 »	450 »	+1 »	13.3 »
49	490 »	500 »	+2 »	15.8 »
53	420 »	435 »	+4 »	16.5 »
Mean	449 g	460 g	+2.4±0.7 %	15.6 mg/100 g

TABLE 11

GUINEA PIGS GIVEN 0.2 MG/KG DIGILAN DAILY. THE TEST TOOK 9 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
36	450 g	450 g	0 %	18.1 mg/100 g
40	520 »	510 »	—2 »	12.7 »
44	465 »	455 »	—2 »	13.8 »
48	380 »	360 »	—5 »	11.7 »
52	500 »	525 »	+5 »	11.2 »
Mean	463 g	460 g	—0.8±1.65 %	13.5 mg/100 g

TABLE 12

GUINEA PIGS GIVEN 1/10 UNIT THYROTROPIC HORMONE DAILY. THE TEST TOOK 9 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
38	410 g	410 g	0 %	15.8 mg/100 g
42	410 »	420 »	+ 2 »	14.3 »
46	420 »	410 »	— 2 »	17.8 »
50	410 »	450 »	+10 »	10.4 »
54	515 »	515 »	0 »	13.0 »
Mean	433 g	441 g	+2±2.1 %	14.3 mg/100 g

TABLE 13

GUINEA PIGS GIVEN 1/10 UNIT THYROTROPIC HORMONE + 0.2 MG/KG DIGILAN DAILY. THE TEST TOOK 9 DAYS

No. of Animal	Weight at Commencement	Weight at End	Change of Weight	Weight of Thyroid
39	465 g	490 g	+5 %	14.2 mg/100 g
43	440 »	470 »	+7 »	15.3 »
47	460 »	480 »	+4 »	18.7 »
51	400 »	425 »	+6 »	15.4 »
55	455 »	475 »	+4 »	19.6 »
Mean	444 g	468 g	+5.2±0.6 %	16.6 mg/100 g

the changes in the body weight of the controls and the digitalis group statistically uncertain. The gain in weight of the animals given thyrotropic hormone was + 2 ± 2.1 per cent on an average, which is roughly the same as in the control group. The dose of thyrotropin was too small to cause any loss of weight, which one might have expected. In the last group where the animals had received thyrotropic hormone + digitalis, the change in body weight was + 5.2 ± 0.6 per cent. Compared with the change in weight in the control group the increase was larger and statistically significant. It was so also in comparison with the digitalis group. It seems that when given together with digitalis, the thyrotropic hormone inhibited the reducing effect of digitalis on weight. — The average weights of the thyroids vary slightly in the different groups, but compared with the control group they are so small that no conclusions can be drawn from them, mainly because of the meagreness of the material.

At autopsy there were no signs indicative of infection in the animals. The amount of perirenal fat varied somewhat in the different animals, but no regularity was noticeable in this respect.

Histology of the Thyroid: The thyroids in the control group did not differ from the control glands described in the foregoing chapters. In this series no essential difference could be noticed between the thyroids in the group given digitalis and the control one. Obviously 9 days had been too short a time for the digitalis to affect the thyroid to any degree that could be observed microscopically. There was very little colloid in the thyroids of the

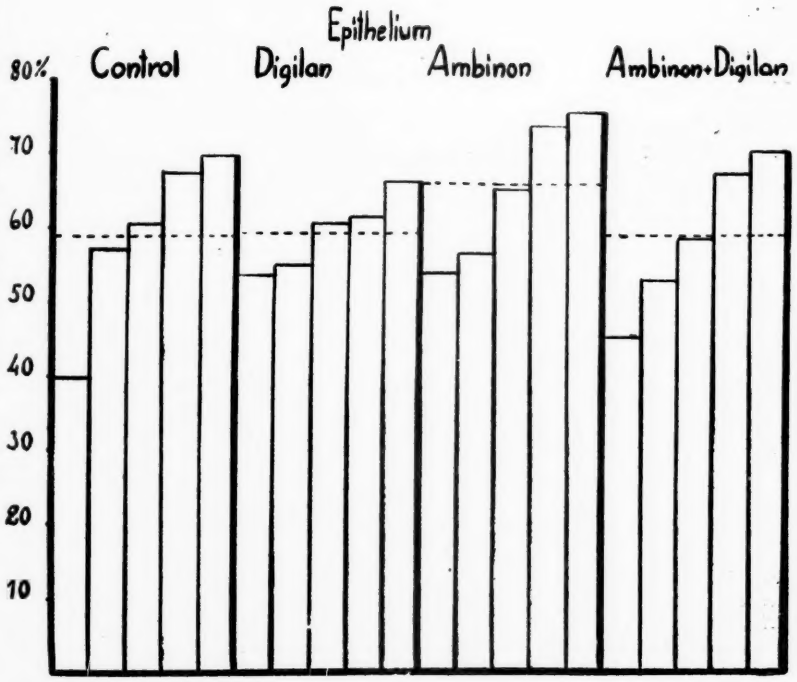


Fig. 13. — Proportions of epithelium represented by columns. Each column corresponds to one thyroid. From left to right: guinea pigs given control solution, 0.2 mg/kg Digilan daily, 1/10 unit thyrotropic hormone, 1/10 unit thyrotropic hormone + 0.2 mg/kg Digilan daily. The substances were injected intraperitoneally for 9 days. The columns are in order of height in each group. The percentages are entered in the ordinate. The means are indicated by broken lines.

animals which had been given thyrotropic hormone. The follicles were of different shapes, some being asteroid, while in some the follicular lumen had become flattened into a narrow linear shape. The epithelium was high, cylindrical. The edge of the colloid showed a number of so-called resorption vacuoles. Droplets of secretion were discernible at the apexes of some cells. The colloid was acidophil in some follicles when stained by Helly-Bensley's and the Azan method, and in others basophil. Larger follicles occurred sometimes in the periphery of the thyroid, with a flat epithelium and colloid staining basophilously red. On an examination of the thyroids of animals which had been given thyrotropic hormone + digitalis a considerably greater amount of colloid was noticed than in the glands of the previous group. The epithelium

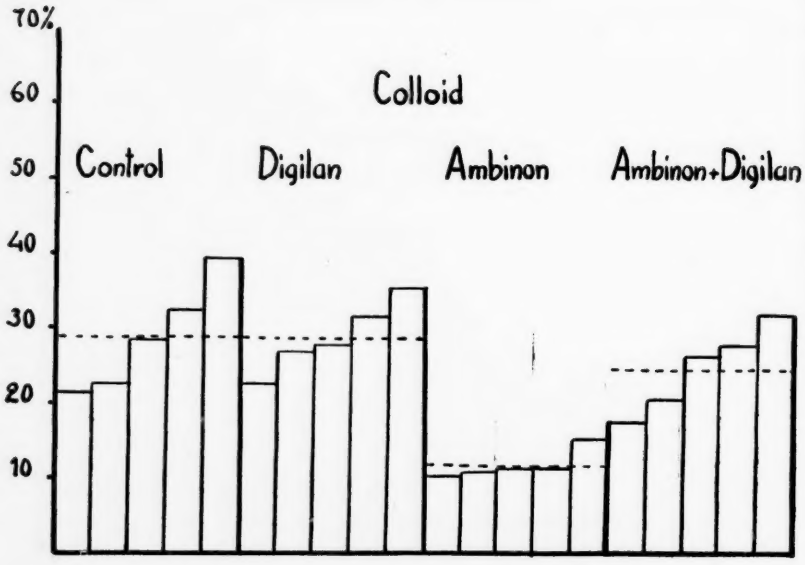


Fig. 14. — Proportions of colloid represented by columns. Each column corresponds to one thyroid. From left to right: guinea pigs given control solution, 0.2 mg/kg Digilan daily, 1/10 unit thyrotropic hormone, 1/10 unit thyrotropic hormone + 0.2 mg/kg Digilan daily. The substances were injected intraperitoneally for 9 days. The columns are in order of height in each group. The percentages are entered in the ordinate. The means are indicated by broken lines.

was comparatively active as a rule, and high as in the preceding group, but there was a greater number of large follicles edged with flattened epithelium which occurred in the central part of the gland also. There was no essential difference in the staining capacity of the colloid compared with the previous group. — The amounts of epithelium and colloid, in percentages, contained by the thyroids in the different groups, are shown in Figs. 13 and 14. The average proportion of epithelium in the control group was 58.7 ± 5.4 per cent, in the group given digitalis 59.1 ± 2.5 per cent, in the thyrotropin group 65.7 ± 4 per cent and in the thyrotropin + digitalis group 58.7 ± 5.2 per cent. The proportion of epithelium was practically the same in all the other groups except the thyrotropin one, in which it seems to have increased slightly. Yet the difference compared with the control group is not statistically significant. — The corresponding proportion of colloid was 28.9 ± 3.3 per cent in the control group, in the group given digitalis 28.8 ± 2.1 per cent, in the thyrotropin group 12.1 ± 0.8 per cent and in

the one in which both thyrotropin and digitalis were administered 24.7 ± 2.6 per cent. There was no noteworthy difference between the control group and the group given digitalis. There was very little colloid in the thyrotropin group. In the group to which both thyrotropin and digitalis was given the amount of colloid was fully twice as large as in the preceding group. The difference is statistically significant.

There seem to be no differences between the amounts of epithelium and colloid in the thyroids even after the administration of digitalis on the 9th day. A comparatively small dose of thyrotropic hormone was injected in this test, but it may yet possibly have increased the proportion of epithelium. It reduced the colloid amount by more than 50 per cent as compared with the controls. Digitalis tended to inhibit this effect of the thyrotropic hormone, and consequently there are no differences in the amounts of epithelium in comparison with the controls. The proportion of colloid in the group which had been given thyrotropic hormone + digitalis approaches the mean value for colloid in the controls.

The series of tests described earlier suggested that some sort of antagonism exists between digitalis and the thyrotropic hormone. Digitalis, when given alone, reduced the body weight of the animals. Although the difference compared with the control group is not statistically significant, the weights obtained in earlier tests seem to point in the same direction. A small amount of thyrotropic hormone, which alone did not affect the weight of the animals to any appreciable degree, inhibited the fall in weight produced by digitalis. The animals which had been given digitalis + thyrotropic hormone had even gained in weight more than the control animals. On the basis of the present small series of tests it is not possible to account for this difference in weight or to decide whether it is in fact a real one or whether it might be due, for instance, to slower peristalsis and a greater accumulation of food remains in the intestines of the animals which had been given digitalis + thyrotropic hormone. Bomskov and his collaborators (1941) also noted that guinea pigs, when given thyrotropic hormone + digitalis, lost less weight than those which had received thyrotropin alone.

The thyrotropic hormone produced a considerable fall in the proportion of colloid while the amount of epithelium increased at the same time. Digitalis tended to inhibit this effect.

DISCUSSION

In the foregoing chapters digitalis has been shown to produce clear signs of inactivation in the thyroid glands of guinea pigs. Their epithelium flattened, the amount of colloid increased, and phenomena pointing to active glandular function in the epithelial cells and in the colloid, such as droplets of secretion and resorption vacuoles, became scarcer. — After nine days of digitalization with sub-toxic doses no changes could be seen but after one month of treatment they were apparent.

The doses of digitalis employed exerted no clear effect on the oxygen consumption of the test animals. It is difficult to bring out small differences in the basal metabolism of animals, for unless they are anaesthetized, it is difficult to keep their metabolism at the basal level. Although the animals may appear motionless, one may well be straining its muscles more than another. Since anaesthesia, when applied frequently, might in itself affect the thyroid and thus disturb the manifestations of the effect of digitalis, it was found to be advisable not to put the animals under anaesthesia while their oxygen consumption was being determined. As was noted in the chapter dealing with oxygen consumption on p. 32, Bomskov and his collaborators (1941) also failed to notice any effect of small digitalis doses on the oxygen consumption of guinea pigs. Nor does digitalis appear to exert any influence on the basal metabolism of normal human beings (Anthony and collaborators 1933).

The blood cholesterol was slightly lower in the digitalis group than in the controls. The difference between the means of the cholesterol values in the two groups was about 14 mg per 100 ml, which was statistically significant. — The cholesterol fell markedly in the group in which digitalis was given, on the first days, whereas the specimens taken 9 days from the beginning of the digitalization

were again on the level of the initial values, and after this the figures fell. The cholesterol values of the control animals also first showed a slight fall and a subsequent rise. Yet compared with the initial values the differences were not equally clear.

The cholesterol content of blood may be affected by a great number of factors and the determination of the blood cholesterol cannot be regarded as any kind of »functional test» specific to the thyroid gland. Peters and Man (1950) studied the significance of the serum cholesterol in diseases of the thyroid by determining, simultaneously, the patients' serum cholesterol and their protein-bound iodine. They inferred that cholesterol determinations are of no help for a diagnosis of thyroid hyperfunction nor in such cases of hypothyroidism as are secondarily due to reduced function of the adrenals or the pituitary gland. Malnutrition may also reduce the cholesterol of the blood (Man and Gildea 1936). — The fact that in the group given digitalis the blood cholesterol was lower than in the control group is not necessarily inconsistent with the effect of digitalis on the thyroid. The digitalis may have lessened the animals' appetite, which seems possible from the fact that the digitalized guinea pigs put on less weight in this series than the controls and that in the others the animals given digitalis usually lost some weight. The poorer nutritional state may have had a share in the decline of the blood cholesterol.

To explain the mechanism of the effect of digitalis on the thyroid gland, another series of tests was performed in which an attempt was made to find out whether digitalis was capable of inhibiting the activating effect of the thyrotropic hormone on thyroid function. It is well known that when the thyrotropic hormone is administered the flat or cuboidal epithelium of a normal thyroid gland is converted into a high and cylindrical one, the number of resorption vacuoles and colloid droplets increases and the amount of colloid declines. In this series of tests we found that $^1/_{10}$ guinea pig units of thyrotropic hormone daily, given for 9 days, produced the expected activation of the thyroids. The epithelium was high, the mean of the epithelial percentages was slightly higher than in the control series, there was an abundance of resorption vacuoles and colloid droplets and the mean of the proportions of colloid was markedly lower than the corresponding value obtained for the controls. At the same time the administration of digitalis

tended to inhibit this activating effect of the thyrotropin. The mean of the epithelial proportions of those guinea pigs which had been given $^1/_{10}$ units of thyrotropic hormone + 0.2 mg/kg Digilan daily for 9 days was the same as that of the controls; the proportion of colloid was clearly double that of the previous group and approached the colloid value for the control group. Some kind of antagonism is also indicated by the fact that, contrary to expectation, the animals which had been given digitalis + thyrotropin did not lose weight, their weight increasing more even than that of the controls.

Yet it remains uncertain whether we have to consider it a real change in weight or whether the difference may not be due to increase of tissular fluid or the fact that intestinal peristalsis may have become slower and the intestinal content thus increased.

The observations made by Bomskov and his collaborators (1941) that small doses of digitalis inhibited the rise in the basal metabolic rate caused by the thyrotropic hormone and reduced the loss in weight occasioned in the animals by this hormone, follow the same trend. It was mainly from these findings that the writers inferred that small doses of digitalis affect the thyroid antagonistically, by inhibiting the stimulating effect of the thyrotropic hormone.

In the series in which digitalis along with the thyrotropic hormone was injected into the test animals, the digitalis seemed to have a tendency of inhibiting the stimulating effect of the thyrotropic hormone on the thyroid gland itself. It would therefore appear that the inactivation caused by digitalis upon the thyroid might take place, at least partly, through inhibition by digitalis of the activating effect of thyrotropin on the thyroid gland.

We noted earlier that small doses of digitalis inhibit the fall in weight caused in animals by thyroxin (Kinsell and collaborators, p. 8) while large ones exert an effect parallel to that of thyroxin, and that small doses counteract the rise in the basal metabolism brought about by the thyrotropic hormone but that large doses act along the same lines as thyrotropin and increase the basal metabolic rate (Bomskov and collaborators, p. 32). Yet from the results obtained in the present work large doses would not seem to activate the thyroid. Although very large doses of digitalis were used in the preliminary tests and the animals died of digitalis intoxication, the thyroids instead of manifesting activity were

remarkably inactive. The doses of digitalis employed for the actual tests themselves were fairly large, though they may still be considered subtoxic. As we have said several times in this report, the thyroids remained inactive in these tests as well. Bomskov and his collaborators (1941) refer to Gremels' studies (1937) and suppose that digitalis might possibly affect the basal metabolism through the medium of acetylcholine. Digitalis, as is known, inhibits the activity of cholinesterase. Large doses of acetylcholine have also been found to increase the basal metabolic rate. The writers continue: »Die grundumsatzerhöhende Wirkung hoher Digitalisdosen kann aber auch die Folge einer Herzschädigung sein. So wird in der Literatur angegeben, dass z.B. bei der Thyreotoxikose eine Grundumsatzsteigerung bis zu 100 % noch als mechanische Folge einer Kreislaufstörung aufgefasst werden kann.»

Although apparently in the tests described earlier in the present study digitalis had an inactivating effect on the thyroid and this effect may possibly take place, at least partly, through the tendency of digitalis to inhibit the stimulating effect of the thyrotropic hormone, no conclusions can yet be drawn from the results so far obtained as to how this inhibitory effect works. Only hypotheses about the working mechanism may be put forward.

Recent research has established the chemical structure of cardiac glycosides. According to textbooks on the subject (*e.g.* Goodman and Gilman 1941 and Oettel 1951) cardiac glycosides are pharmacologically made up of active aglycone and one or more sugar molecules combining with it. The sugars are different monosaccharides. The aglycones may be liberated from the heart glycosides by acids or enzymatic hydrolysis.

All aglycones known have a nucleus of cyclo-pentano-perhydrophenanthrene. They have this nucleus in common with cholesterol, bile acids, sexual hormones and the hormones of the adrenal cortex. It is interesting to note, according to different authorities, that many of these substances also affect the thyroid.

A great amount of work has been done to elucidate the interrelation between the thyroid, the pituitary gland and the sexual glands. We mention only that folliculin (oestrogen) is supposed to inhibit the effect of the thyrotropic hormone on the thyroid (Aron and Benoit 1932) and to increase the amount of colloid in the thyroid (Karp and Kostkiewicz 1934). Folliculin inhibits the

elevation of the rate of basal metabolism caused by the thyrotropic hormone in rats whose pituitary gland has been removed (Gessler 1937). Large amounts of oestrogen probably diminish the thyrotropic secretion of the pituitary gland (Gessler 1937). Synthetic oestrogens, derivatives of stilbestrol, which differ from natural oestrogens in composition, have similar effects. Chamorro (1949) and Calapà (1950) noted that they inhibit the goitrogenic effect of thiouracil-derivatives. Thiouracil has the property of preventing the entrance of iodine into the thyroid, which makes the formation of thyroid hormone impossible. Lack of thyroid hormone, on the other hand, increases the secretion of thyrotropin, which results in hyperplasia of the thyroid gland. On this hyperplasia synthetic oestrogens are supposed to have an inhibitory effect. So far all attempts to account for the ultimate cause of these oestrogenic effects have been resultless.

Gumbrecht and Loeser's (1938) discovery that the corpus luteum hormone, in contrast to oestrogens, exerts no effect on the thyroid, is worth noting.

Testosterone again is known to have a slightly activating effect on the thyroid (Carrière and collaborators 1938, Selye 1939).

Some kind of antagonism is likewise present between the cortical portion of the adrenal and the thyroid hormone. Thorn found that the extract from the adrenal cortex lessened the emaciation of the rats which had been fed with thyroxin, nor did the weights of their hearts increase as much as the heart weights of the animals which had been given thyroxin alone. Kinsell and his collaborators (1942), as has been stated earlier, found that the extract from the adrenal cortex and desoxycorticosterone also inhibited the emaciation of animals produced by thyroxin. Elmer and his collaborators (1935) again declared that »Cortin» has no effect whatever on the histologic picture of the thyroid gland nor does it exert any inhibitory effect on the hormone of the anterior lobe of the pituitary gland which activates the thyroid. Elmer and his collaborators (1939) noted that desoxycorticosterone acetate stimulated the thyroid slightly and that DOCA when given in animal experiments was incapable of inhibiting the effect of the thyrotropic hormone.

Interesting studies have also been made on the reciprocal effect of cholesterol and the thyroid. Saegesser (1933) fed tadpoles with cholesterol dissolved in olive oil and thyroxin contemporaneously and noted that cholesterol inhibited the effect of thyroxin in

accelerating metamorphosis and preventing growth. — Marx and his collaborators (1948) found that if rats were given great amounts of cholesterol with their food, when they were simultaneously fed with toxic doses of thyroxin, this prolonged their lifetime considerably. Winebrenner and Marx (1949) declared that cholesterol reduced the basal metabolic rate of hyperthyroid rats.

If we compare the structural formulae of the different steroids which counteract the thyroid (cholesterol, oestrogen and the heart glycoside aglycones experimented with in the present work), we notice as a common feature the cyclo-pentano-perhydro-phenanthrene nucleus, the hydroxyl group in position C_3 and in C_{13} a methyl group. The double bonds in the nucleus and the side chain in C_{17} vary considerably. When comparing with each other those steroids which had no effect on the thyroid or a slightly stimulating one (progesterone, testosterone and desoxycorticosterone), we note as a common property the same cyclo-pentano-perhydro-phenanthrene nucleus with a double bond between C_4 and C_5, an oxygen atom in position C_3 in an oxo-form, and a methyl group in C_{13}. On the other hand, the side chain in C_{17} varies. In a comparison of the two groups we find that they have a methyl group in common in C_{13}. It seems remarkable that all the steroids which affected the thyroid antagonistically had an OH group in position C_3 while those which produced no effect or perhaps a slightly stimulating one had oxygen only in the corresponding atom (pp. 50—52).

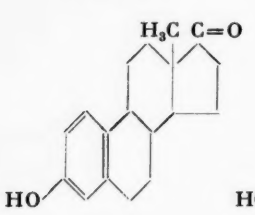
Cholesterol is known to be capable of conversion into ester and this esterification takes place through the group of hydroxyls in position C_3. — Saegesser (1933) when studying the protective effect of cholesterol against thyroxin, considered an ester-like combination of cholesterol with the carboxyl group in thyroxin possible.

Our knowledge of the thyrotropic hormone of the anterior lobe of the pituitary gland is still incomplete. In his monograph on the thyroid, Means (1948) states that the only thing known of its structure is that it is a protein and that in activating the thyroid it itself obviously becomes inactive, yet is not destroyed, its activity being restored by reducing substances.

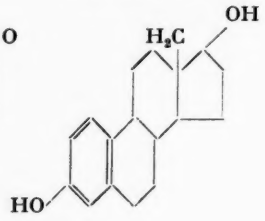
We might now presume some kind of incorporation of digitalis and the thyrotropic hormone into one and that the complex would be void of the stimulating effect possessed by thyrotropin alone. It is not impossible that the hydroxyl group in C_3 might have some

STEROIDS WITH AN INHIBITORY EFFECT ON THE THYROID

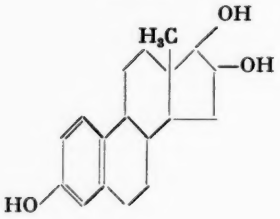
Oestrone



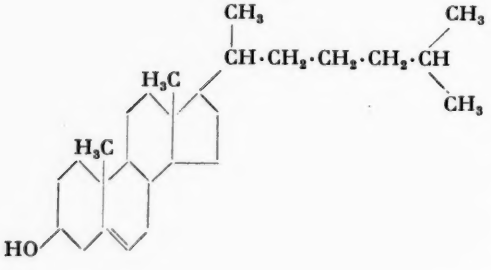
Oestradiol



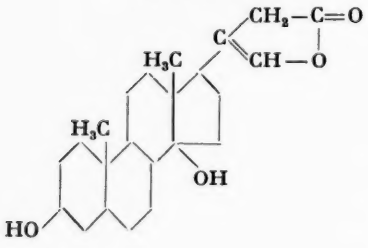
Oestriol



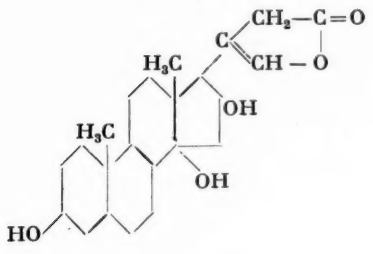
Cholesterol



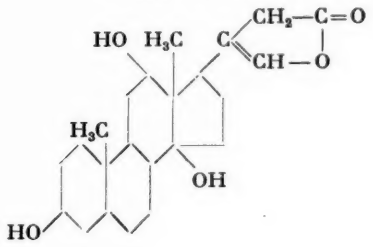
Digitoxigenin



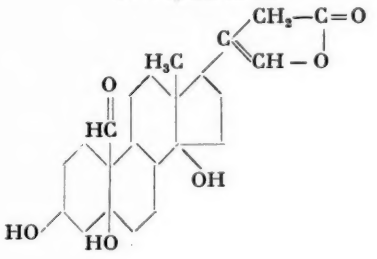
Gitoxigenin



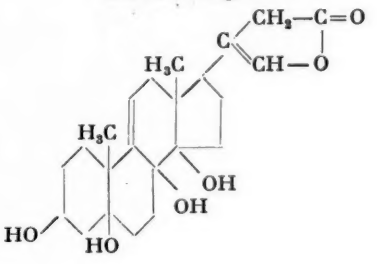
Digoxigenin



k-Strophanthidin

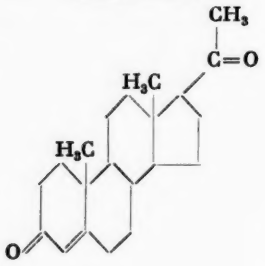


Convallatoxigenin

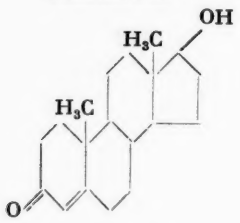


STEROIDS WITH NO EFFECT ON THE THYROID OR A SLIGHTLY ACTIVATING ONE

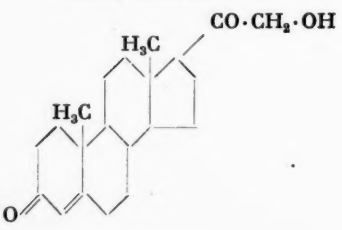
Progesterone



Testosterone



Desoxycorticosterone



share of its own in this. In its capacity as a protein the thyrotropic hormone should contain at least one carboxyl group of amino acid and consequently a kind of incorporation resembling that of esters might possibly be suggested.

There has been a tendency, of late, to explain various phenomena on the basis of the General-Adaptation-Syndrome. It might indeed be suggested that the guinea pigs which in these tests were given relatively large continued doses of cardiac glycosides may have been under the continuous influence of stress. No systematical work has been done on the effect of continuous stress upon the thyroid (Selye 1950), but it is possible that the thyroid might

under these conditions become inactivated through the mediation of the adrenal gland. ACTH and cortison, when administered over a long time, are known to reduce the amount of serum-protein bound iodine (Hardy and collaborators 1950). Cortison diminishes the capacity of the thyroid to bind radioactive iodine and often produces »an elevation of the renal plasma I^{131} clearance rate» (Berson and Yalow 1952).

Further, it is not impossible that digitalis may exert a non-specific effect on the thyrotropin secretion of the pituitary gland by reducing it.

Some suggestions only have been put forward above as to the manner in which cardiac glycosides might have an inactivating effect upon thyroid function. Obviously a great amount of research is still necessary before such a complicated problem as the effect of digitalis on the thyroid can be cleared up.

SUMMARY

The effect of the following cardiac glycosides on the thyroid was studied: A mixture of the crystalline lanatosides A, B, and C (»Digilan Orion»), digitoxin (»Digitoxin Star»), k-strophanthin (»k-Strophanthin Orion») and convallatoxin (»Convallatoxin Roche»).

One hundred and thirteen guinea pigs were used for the tests, mainly males, which have no cyclic hormonal fluctuation as have the females, capable of unsettling a study of the kind conducted. Only one series of 12 guinea pigs were females.

To disclose any digitalis intoxication, an electrocardiogram was taken of 10 guinea pigs after treatment with digitalis and of 58 guinea pigs before and after treatment. Besides the usual limb leads, 5 unipolar chest leads were placed. The electrocardiograms showed no pathological changes.

The thyroid function was estimated from histological preparations. The proportions of the thyroid epithelium and colloid were measured in all the series of tests according to the Uotila—Kannas method. In one series the average height of the epithelial cell was measured, besides.

In the series in which 10 guinea pigs had been given 1 mg/kg Digilan by mouth daily for 60 days, and 10 control solution, the thyroids of those which received Digilan appeared less active, the epithelium was flatter and there was more colloid. The average height of the epithelium cell was $4.7 \pm 0.6\ \mu$ in the Digilan group and $6.1 \pm 0.5\ \mu$ in the control group. The proportion of epithelium was 41.9 ± 3 in the Digilan and 64.8 ± 1.8 in the control group. The corresponding values for colloid were 50.5 ± 2.7 and 22.4 ± 2.7 per cent. The differences between the means of the proportions of epithelium and those between the means of the proportions of colloid were highly significant.

Six of the 12 female guinea pigs were given control solution

intraperitoneally and 6 0.2 mg/kg Digilan daily for 60 days. In the controls the mean of the epithelium proportions was 62.3 ± 1.5 per cent, and for the animals which were given Digilan 48.8 ± 3 per cent. The controls had 32.8 ± 1.8 per cent colloid on an average and the guinea pigs given Digilan 46.9 ± 2.8 per cent. The difference between the means is statistically significant both for epithelium and colloid.

Changes with a similar trend were noted in the thyroids of the guinea pigs which had been given intraperitoneal injections of digitoxin, k-strophanthin and convallatoxin for 30 days. The series consisted of 40 guinea pigs, of which 10 were given control solution, 10 digitoxin, 10 k-strophanthin and 10 convallatoxin daily. The proportion of epithelium was 63.9 ± 1.8 per cent on an average in the controls, 57.2 ± 1.8 in the digitoxin group, 54.3 ± 2.4 in the k-strophanthin group and 59.7 ± 1.8 in the convallatoxin group. Compared with the control group the digitoxin and k-strophanthin groups showed a significant difference, while there was no statistical difference in the convallatoxin group. For colloid the corresponding proportions were as follows: Control group 21.2 ± 2.7, digitoxin group 28.6 ± 1.2, k-strophanthin group 31.5 ± 1.1 and convallatoxin group 29.4 ± 1.9 per cent. In comparison with the controls, the difference is significant in the k-strophanthin group, and in the digitoxin and convallatoxin groups almost significant.

The effect of digitalis on the oxygen consumption and blood cholesterol was studied on 10 guinea pigs, 5 of which were given control solution and 5 Digilan by mouth for 70 days. The daily dose of Digilan was 1 mg/kg for the first 30 days and 2 mg/kg for the rest of the time. Twelve oxygen consumption and blood cholesterol determinations were carried out within this time on each guinea pig. There were no statistically significant differences in the values for oxygen consumption between the controls and the Digilan group. The blood cholesterol of the guinea pigs given Digilan seemed to be lower than that of the controls. Before administration of Digilan 2 determinations were made on each guinea pig, so that 20 normal values were obtained. Their mean was 163 ± 4.5 mg per 100 ml. The mean of the blood cholesterol values during the test was 163.6 ± 2.6 mg per 100 ml for those which had been given control solution and 149.2 ± 2.4 mg per 100 m lfor the animals given Digilan.

The difference compared with the mean for the controls is 14.4 mg per 100 ml, which is statistically significant. The amount of epithelium was 54.4 ± 4.2 per cent on an average in the controls and in the Digilan group 42.6 ± 3.2 per cent. The corresponding values for colloid were 20 ± 1.8 per cent in the controls and 41.5 ± 2.1 per cent for the guinea pigs which had been given Digilan.

Five guinea pigs were injected intraperitoneally with control solution on 9 days, 5 with Digilan, 5 with thyrotropic hormone (»Ambinon Organon») and 5 with thyrotropic hormone + Digilan (the daily Digilan dose was 0.2 mg/kg and that of thyrotropic hormone $^1/_{10}$ guinea pig units). Digilan and the thyrotropic hormone were found to counteract each other in some way. There were small differences in the body weights of the animals in the different groups. In the control group the change in weight was + 2.4 ± 0.7 per cent, in the Digilan group —0.8 ± 1.6, in the thyrotropin group + 2.0 ± 1.1 and in the thyrotropin + Digilan group + 5.2 ± 0.6 per cent. In this series the thyroids of the controls and the Digilan group showed no difference. Obviously 9 days was too short a time for Digilan to affect the thyroid. In the thyroids of the animals given thyrotropic hormone the epithelium was active, high and cylindrical; there was very little colloid. Compared with them the thyroids of those which were given thyrotropic hormone+ Digilan appeared less active. The mean of the proportion of epithelium was 58.7 ± 5.4 in the controls, in the Digilan group 59.1 ± 2.5, in the thyrotropin group 65.7 ± 4 and in the thyrotropin + Digilan group 58.7 ± 5.2 per cent. For colloid, the corresponding values were 28.9 ± 3.3 per cent in the control group, 28.8 ± 2.1 in the Digilan group, 12.1 ± 0.8 in the thyrotropin group and 24.7 ± 2.6 per cent in the thyrotropin + Digilan group. There was more than twice as much colloid in the thyroids of the guinea pigs given thyrotropic hormone + Digilan than in the thyroids of those which had received thyrotropic hormone alone. The difference between the means for the two lastmentioned groups is statistically significant.

To sum up and in reply to the questions put it can be noted that

1. The cardiac glycosides employed, digilan (mixture of lanata glycosides), k-strophanthin, digitoxin and possibly also convallatoxin, produced changes in the histological picture of the thyroid in the tests carried out.

2. The thyroids appeared inactive, the epithelium seemed to have flattened, the proportion of epithelium diminished and that of colloid increased.

3. The administration of lanata glycosides did not produce clear differences in the oxygen consumption of the test animals, but it had a slightly reducing effect on the blood cholesterol.

4. It seems that the inactivating effect of cardiac glycosides on thyroid function, partly at least, takes place by way of inhibition of the activating influence exerted by the thyrotropic hormone on the thyroid.

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